

PATENT APPLICATION  
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Hiroyuki HIRAI, et al.

Appln. No.: Not Yet Assigned

Confirmation No.: Not Yet Assigned

Group Art Unit: Not Yet Assigned

Filed: February 22, 2002

Examiner: Not Yet Assigned

For: METHOD OF IDENTIFYING PROPERTIES OF SUBSTANCE WITH RESPECT TO HUMAN  
PROSTAGLANDIN D RECEPTORS

PRELIMINARY AMENDMENT

Commissioner for Patents  
Washington, D.C. 20231

Sir:

Prior to examination, please amend the above-identified application as follows:

**IN THE SPECIFICATION:**

**Page 12, please delete the second full paragraph and replace it with the following new paragraph:**

Instead of the aforementioned recombinant human CRTH2-related protein, human tissue, a human cell strain, etc. in which natural human CRTH2 is highly expressed may be used in the present identification method. Alternatively, a transformant which has been transformed by a gene encoding the aforementioned human CRTH2-related protein may be used. In such a human tissue, human cell strain, etc., preferably, only human CRTH2 is expressed, and the DP receptor and other prostanoid receptors are not expressed.

**Page 15, please delete the first full paragraph and replace it with the following new paragraph:**

When a selective modulator with respect to activation of human CRTH2, etc. is identified by means of the present identification method, no absolute limits are imposed on a test substance. Briefly, in the present identification method, a test substance may be a naturally occurring product (including a recombinant protein produced through biotechnological technique) or a chemically synthesized product.

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When the present identification method is carried out, if necessary, a known labeled or unlabeled ligand (for example, prostanoid such as PGD<sub>2</sub>) may be used.

**Page 18, please delete the first full paragraph and replace it with the following new paragraph:**

As described above, the properties of a test substance with respect to prostaglandin D or a human prostaglandin D receptor are identified by correlating the effect of the substance on human CRTH2 (for example, a selective modulator effect) with the effect of the substance on the human prostaglandin D receptor. When the identification method is used for, for example, screening of drugs, it can greatly contribute to the relevant industry.

**Please delete the paragraph bridging pages 20, 21 and 22 and replace it with the following new paragraph:**

Each of KB8 cells, KD36 cells, and K562/neo cells was resuspended in Hank's balanced salt solution (HBSS, product of Gibco BRL) so as to attain a concentration of  $3 \times 10^7$  cells/ml. The resultant suspension (0.1 ml) was placed in a 0.5-ml microtube, and then cooled on ice. Subsequently, 1 nM of [<sup>3</sup>H] PGD<sub>2</sub> (product of Amersham) which had been diluted with HBSS was added to the suspension, to thereby allow reaction to proceed on ice for one hour. The reacted cells were placed carefully onto RPMI1640 medium (1 ml) containing 1 M sucrose and 10% fetal bovine serum, the medium having been placed in an 1.5-ml microtube and cooled by ice, and subjected to centrifugation (10,000 revolutions, three minutes) by use of a micro-centrifuge. After the supernatant was aspirated from the tube such that the mixture (about 0.1 ml) remained in the tube, the mixture was further subjected to centrifugation (10,000 revolutions, one minute) such that the reaction mixture did not remain on the tube wall, and subsequently the supernatant was removed as carefully as possible so as to avoid removing the cells. The radiation activity of the cells bound to [<sup>3</sup>H] PGD<sub>2</sub> was measured by use of a liquid scintillation counter. The radiation activity of the cells when measured, in a manner similar to that described above, in the presence of unlabeled PGD<sub>2</sub> (concentration: 200 times or more that of [<sup>3</sup>H] PGD<sub>2</sub>) was used as an index of non-specific binding. As a result, as shown in Fig. 1, the specific binding of [<sup>3</sup>H] PGD<sub>2</sub> to K562/neo is not observed. In contrast, the specific binding of [<sup>3</sup>H] PGD<sub>2</sub> to KB8 or KD36 is observed. In this measurement system, anti CRTH2 antibody BM7 (Nagata, K. et al., J. Immunol., 162: 1278-1286, 1999 and Nagata, K., et al., FEBS Lett., 459: 195-199, 1999) selectively inhibited the binding of [<sup>3</sup>H] PGD<sub>2</sub> to KB8 in a concentration-dependent manner. The results show that this method can identify a selective modulator with respect to human CRTH2, which does not act on the DP receptor.